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SPECIFICITY IN STABILIZING SUSPENSIONS OF RICKETTSIA RICKETTSII

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ABSTRACT

The infectivity of suspensions of partially purified Rocky Mountain spotted fever rickettsiae was maintained at a high level during storage at 4 C for 1 week or longer by 0.1 M glutamine, but only for much shorter periods by lower concentrations (0.05 M, 0.01 M) of this compound. This stabilization may be related to the prevention of loss of metabolically important glutamate from these organisms, or to a stabilization of the structure of the rickettsiae, or to both. In any case, there appears to be a specificity of chemical structure required for stabilization. Glutamine and glutamate were outstandingly effective, asparagine provided some stability but of a much lower level, and other compounds tested that have no similarity to glutamine, such as inositol and thiourea, were completely ineffective. The stabilizing effect of glutamine on partially purified rickettsiae was enhanced greatly by the addition of 0.3 per cent bovine albumin. Crude yolk-sac suspensions of Rickettsia rickettsii were not stabilized by 0.1 M glutamine; however, addition of both bovine albumin and glutamine to yolk-sac samples overcame the poor stability of the rickettsiae in this type of suspension. It appears that only certain types of protein can produce conditions favorable for maintenance of rickettsial infectivity in yolk-sac preparations.

SPECIFICITY IN STABILIZING SUSPENSIONS OF RICKETTSIA RICKETTSII

In studying the stability of the rickettsia of Rocky Mountain spotted fever, Rickettsia rickettsii, we observed that (i) under certain conditions purified or partially purified rickettsial suspensions were more stable to storage at 4 C than crude yolk-sac suspensions of the organism, and (ii) the requirement for glutamine or glutamate to stabilize partially purified rickettsiae appeared to be specific for these compounds.

This stabilizing effect of glutamate was not unexpected since Bovarnick and co-workers[1] had shown a requirement for 0.005 M glutamate to maintain purified typhus rickettsiae in metabolically active condition for several hours at 30 to 35 C, or for 24 hours at 0 C. However using partially purified spotted fever rickettsiae we have obtained somewhat better results with glutamine than with glutamate. Also, we have observed a sharp difference in the stabilizing effect of these compounds at the 0.1 M and 0.05 M levels and only a slight stabilizing effect of glutamine for these organisms in crude yolk-sac suspensions.

This manuscript presents the results of studies on the survival of R. rickettsii in crude and partially purified suspensions.

Partially purified suspensions of spotted fever rickettsiae employed in these studies were prepared by homogenizing infected yolk sacs in potassium phosphate buffer at pH 7.5 (hereafter referred to as K7.5) and applying one cycle of low and high speed centrifugation to sediment the organisms from the soluble yolk-sac environment. Rickettsial sediments resuspended in K7.5 buffer were used as the partially purified preparations in these tests. Compounds being tested were dissolved in K7.5 buffer, and the final pH was adjusted to 7.5 for the mixture of rickettsiae and stabilizer. Aliquots of the rickettsial suspension and of the solution of the compound being tested were mixed in equal volumes and stored at 4 C in 5-ml, rubber-stoppered glass bottles. Samples were removed at intervals and assayed by yolk-sac inoculation of 7-day embryonated eggs.

The stabilizing effects of glutamine and glutamate on the infectivity of partially purified R. rickettsii are shown in Table 1. Control suspensions in K7.5 buffer showed large decreases in titer in 1 day at 4 C (as indicated in the top line), whereas rickettsiae in the presence of 0.05 M, or 0.1 M glutamine or glutamate possessed 7 logs or more of infectivity at that time. At 6 days samples containing less than 0.1 M stabilizer had no titer but those with 0.1 M glutamine still retained more than 6 logs of infectivity. Glutamine appeared to be more effective than glutamate for stabilizing the rickettsiae. Note, for any concentration, that samples with glutamine had higher titers than samples with glutamate.

TABLE 1. STABILIZING EFFECT OF GLUTAMINE AND GLUTAMATE ON THE INFECTIVITY OF PARTIALLY PURIFIED SUSPENSIONS OF RICKETTSIA RICKETTSII AT 4 C

Stabilizer	Infectivity Titer[a]/ days at 4 C			
	0	1	3	6
None	7.8	2.7	0	0
Glutamine 0.10 M	7.8	8.1	7.3	5.3
Glutamine 0.05 M	7.7	7.8	6.3	0
Glutamine 0.005 M	7.7	7.1	5.9	0
K-glutamate 0.10 M	7.7	7.8	7.2	5.4
K-glutamate 0.05 M	7.7	7.4	5.7	0
K-glutamate 0.005 M	7.7	6.9	3.3	0

a. Log_{10} yolk-sac LD_{50} per ml.

Spotted fever rickettsiae in yolk-sac suspensions were not stabilized effectively by 0.1 M glutamine, contrary to the stabilization obtained with partially purified preparations. This difference is shown in Figure 1. The curves represent the average loss of infectivity observed with partially purified and crude suspensions in several experiments. Note the very pronounced difference in the stability of the purified suspensions in the presence and absence of 0.1 M glutamine. In some tests yolk-sac samples containing glutamine lost infectivity more rapidly than control samples; in other tests the reverse was true.

Figure 2 depicts the greater stabilization of partially purified rickettsiae obtained with a combination of 0.1 M glutamine and 0.3% bovine serum albumin than with 0.1 M glutamine alone. Curves shown represent the average values of seven experiments. At 8 days, samples containing both stabilizers had infectivity titers 1 log higher than samples with glutamine only. At 12 days, this difference had increased to 2 logs, and at 20 days, to 4 logs. Loss of infectivity by samples to which 0.3% bovine albumin alone was added was similar to that of samples that contained glutamine alone.

As previously mentioned, crude yolk-sac preparations of R. rickettsii were stabilized only slightly by glutamine. However, as shown in Figure 3 the addition of a combination of 0.1 M glutamine and 0.3% bovine albumin

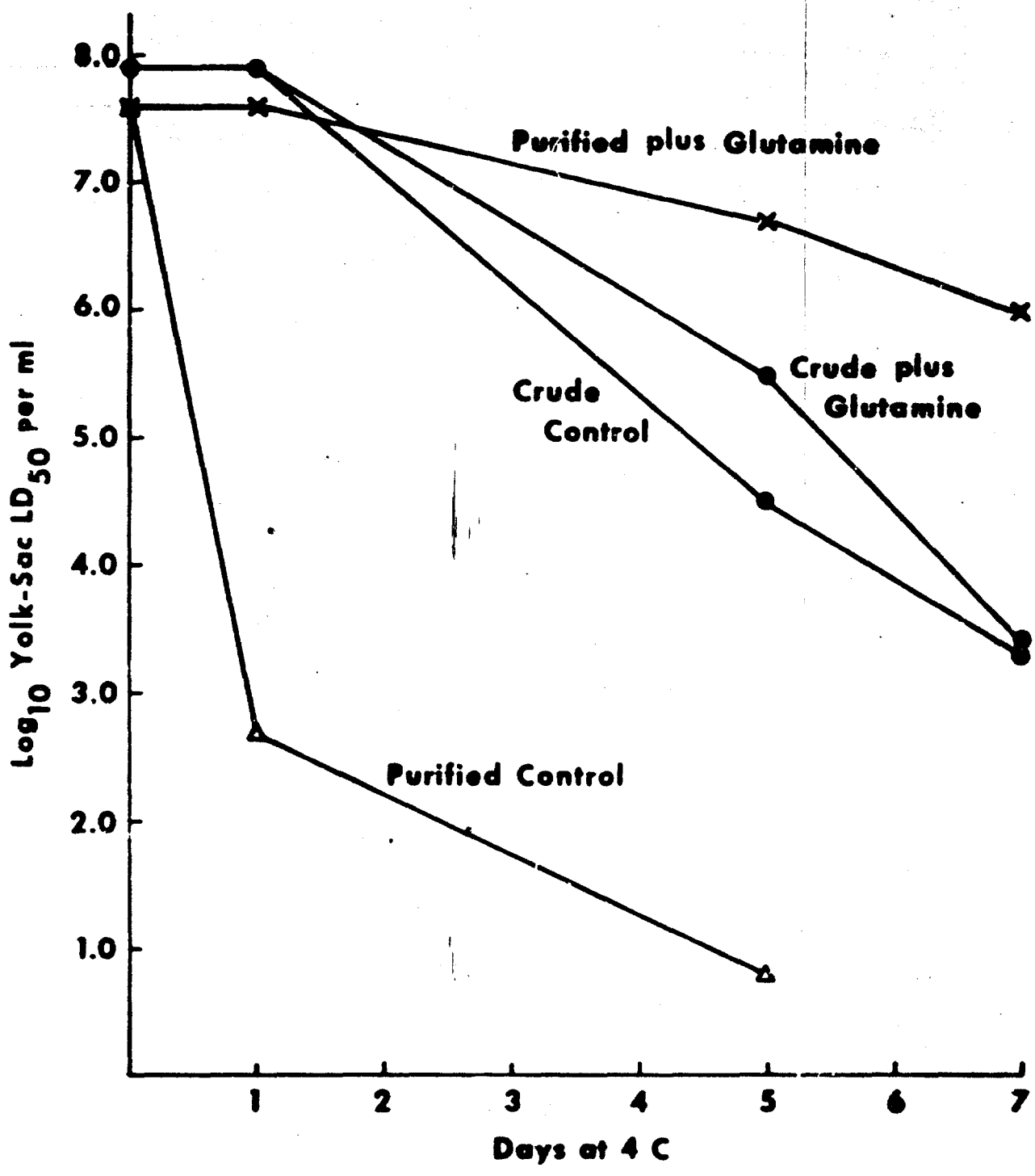

Figure 1. Comparison of the Effect of 0.1 M Glutamine on Partially Purified and Crude Yolk-Sac Suspensions of Rickettsia rickettsii.

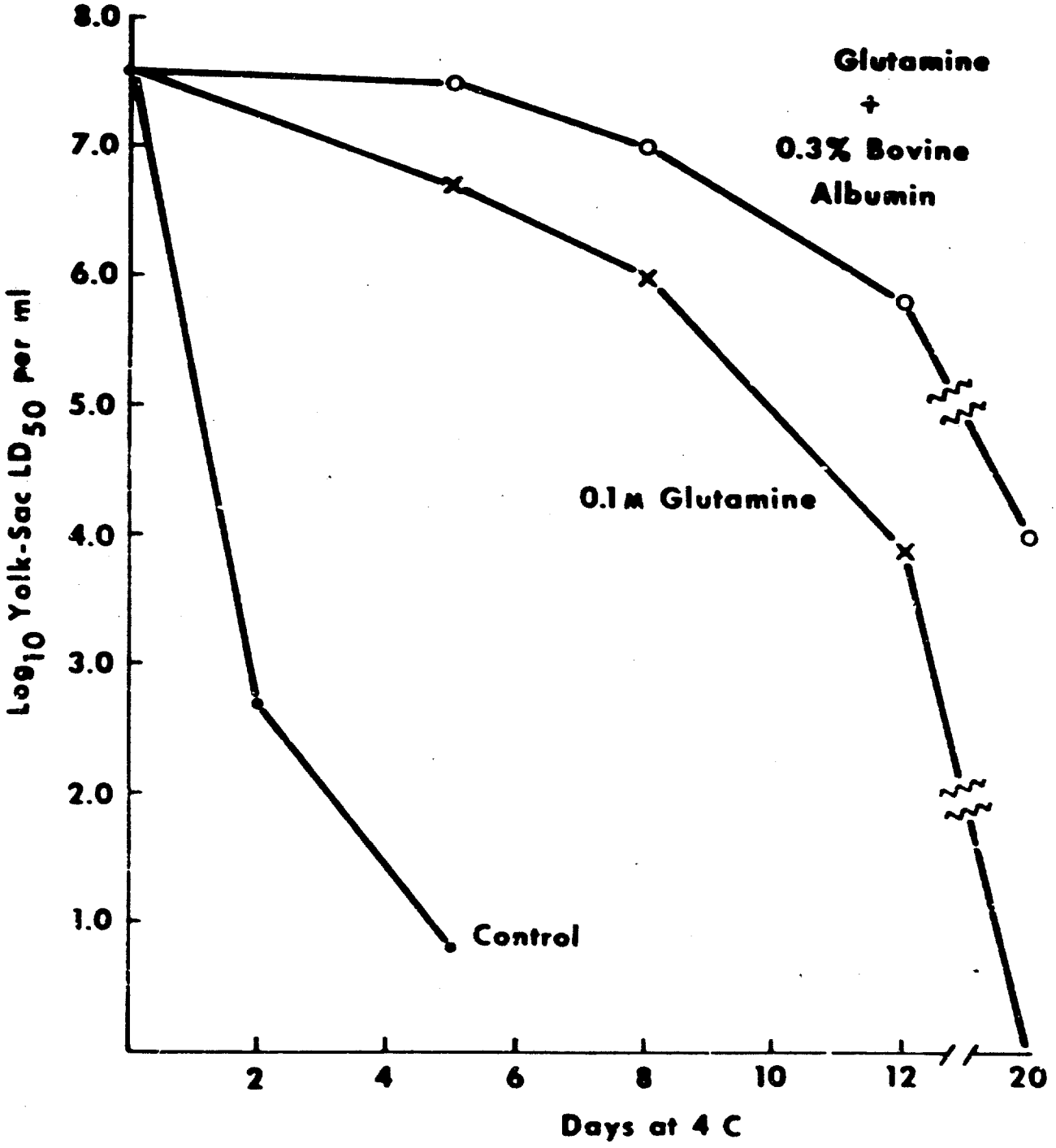

Figure 2. Effect of Bovine Albumin on the Glutamine Stabilization of Partially Purified Rickettsia rickettsii.

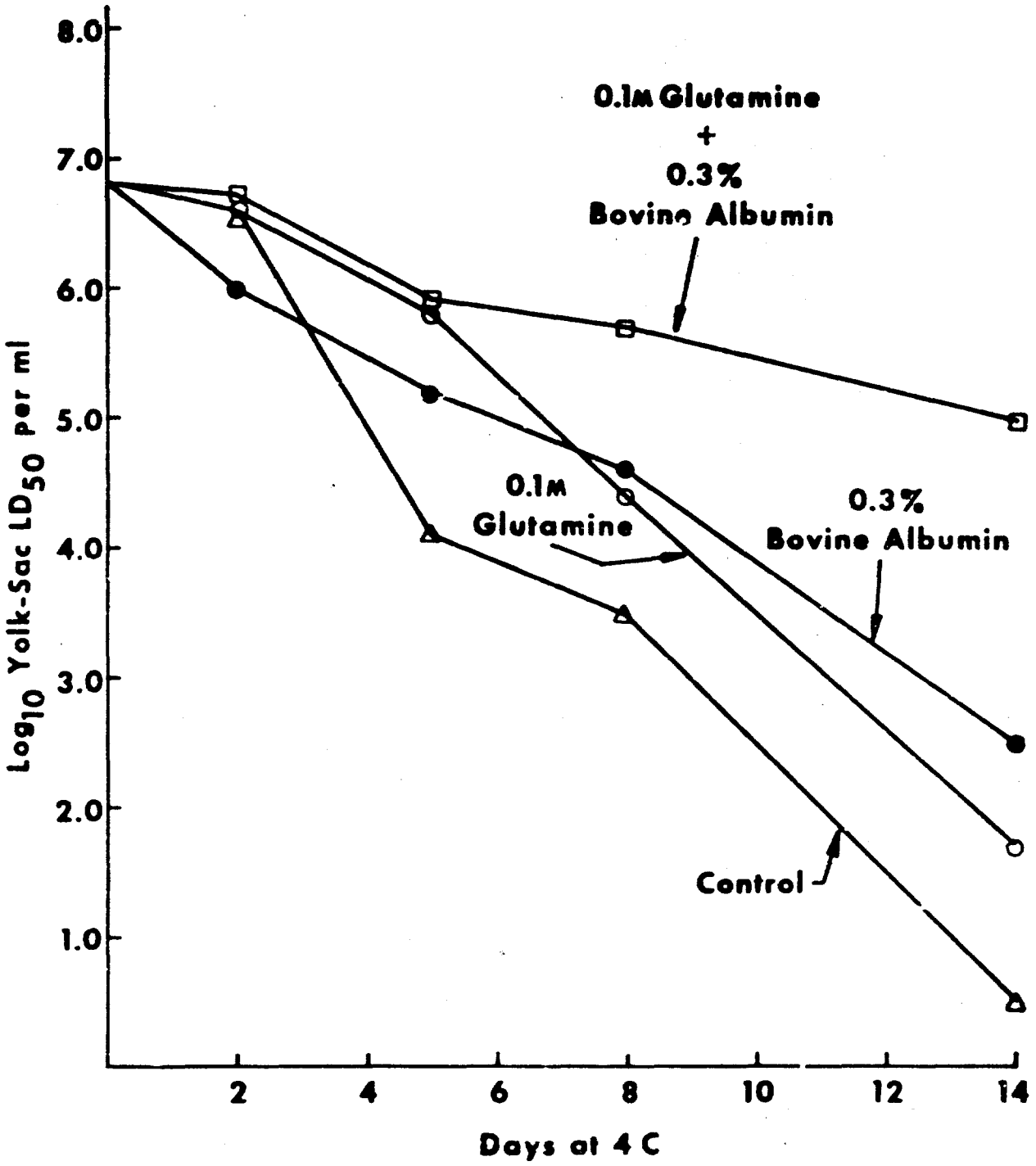

Figure 3. Effect of Combining Glutamine and Bovine Albumin on the Stabilization of Yolk-Sac Suspensions of Rickettsia rickettsii.

to this preparation was effective in maintaining rickettsial infectivity at a relatively high level for 10 to 12 days. Glutamine alone and bovine albumin alone were not nearly as effective as the combination of the two substances.

We have observed that only certain proteins, or certain types of proteins, are effective in maintaining the infectivity of R. rickettsii in yolk-sac suspensions. The difference in stabilizing effect of several proteins with this type of preparation is indicated in Table 2. Although the proteins were tested both alone and in the presence of 0.1 M glutamine, Table 2 shows the results obtained with the proteins in combination with glutamine. Glutamine alone and bovine albumin alone were relatively ineffective as stabilizers, but as shown in this table the combination of the two provided good stability. No stabilization was obtained with egg albumin or gelatin, even in combination with glutamine. Casein was also tested and found to lack any stabilizing capacity.

Recently, two proteins from human serum, a β-lipoprotein and an α-globulin have been found to stabilize these rickettsiae in yolk-sac samples more completely than bovine albumin. Note that the combination of β-lipoprotein and 0.1 M glutamine maintained the original infectivity titer for two weeks.

TABLE 2. COMPARISON OF THE EFFECT OF DIFFERENT PROTEINS ON THE INFECTIVITY OF YOLK-SAC SUSPENSIONS OF RICKETTSIA RICKETTSII

Proteins[a]	Infectivity, $\log_{10}$ yolk-sac LD_{50}/ml days at 4 C		
	8	14	24
Control (0.1 M glutamine)	4.4	1.7	0
0.1 M glutamine plus			
Bovine albumin	5.7	5.0	2.0
Egg albumin	4.3	2.1	0
Gelatin	3.7	0.5	0
Human α-globulin	6.2	5.2	3.5
Human β-lipoprotein	6.5	6.4	4.5

a. Proteins at 0.3% concentration were in combination with 0.1 M glutamine.

Bovarnick and Snyder[1] showed that glutamate was the chief substrate of typhus rickettsiae. We have found that glutamine is somewhat more effective than glutamate for stabilizing spotted fever rickettsiae. Huang and Weiss[2] of the Naval Medical Research Institute have shown in metabolic studies that R. quintana utilizes glutamine to a much greater extent than glutamate. In preliminary studies, Mr. Rees of our laboratory, and Dr. Weiss have obtained a similar result with R. rickettsii.

We do not know whether the stabilization of spotted fever rickettsiae by glutamine that we have observed is related to a metabolic function of the compound. The much more pronounced stabilization obtained with 0.1 M glutamine, as compared to 0.05 M, and the fact that glutamine was shown by Wachter and Comer[3] to stabilize eastern equine encephalitis virus suggest that glutamine may be acting in a dual manner. First, it may be stabilizing in some way because of its metabolic function. Secondly, it may be helping to maintain the structural integrity of the rickettsiae by a mechanism similar to the glutamine stabilization of the eastern equine virus.

With regard to the stabilization of this rickettsia in yolk-sac preparations, the indicated specificity for serum proteins in this stabilization may be associated with the known interaction of serum albumins and globulins with fatty acids, sterols, and phospholipids, any of which may be detrimental to rickettsial infectivity.[4-6]

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